

SEQUENCE LISTING

```
<110> RANGANATHAN, ANAND
<120> DNA MANIPULATION METHODS AND APPLICATIONS FOR SYNTHETIC
      ENZYMES
<130> SHW-009US
<140> 10/009,873
<141> 2002-11-1
<150> PCT/GB00/02286
<151> 2000-06-12
<150> GB 9913694.7
<151> 1999-06-11
<160> 30
<170> PatentIn Ver. 3.3
<210> 1
<211> 12
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 1
                                                                    12
aaatctagac cc
<210> 2
<211> 12
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 2
                                                                    12
agatctagac cc
<210> 3
<211> 374
<212> PRT
<213> Streptomyces lividans
<400> 3
Met Ala Gly Thr Asp Arg Glu Lys Ala Leu Asp Ala Ala Leu Ala Gln
Ile Glu Arg Gln Phe Gly Lys Gly Ala Val Met Arg Met Gly Asp Arg
Thr Asn Glu Pro Ile Glu Val Ile Pro Thr Gly Ser Thr Ala Leu Asp
```

		35					40					45			
Val	Ala 50	Leu	Gly	Val	Gly	Gly 55	Ile	Pro	Arg	Gly	Arg 60	Val	Val	Glu	Val
Tyr 65	Gly	Pro	Glu	Ser	Ser 70	Gly	Lys	Thr	Thr	Leu 75	Thr	Leu	His	Ala	Val 80
Ala	Asn	Ala	Gln	Lys 85	Ala	Gly	Gly	Gln	Val 90	Ala	Phe	Val	Asp	Ala 95	Glu
His	Ala	Leu	Asp 100	Pro	Glu	Tyr	Ala	Lys 105	Lys	Leu	Gly	Val	Asp 110	Ile	Asp
Asn	Leu	Ile 115	Leu	Ser	Gln	Pro	Asp 120	Asn	Gly	Glu	Gln	Ala 125	Leu	Glu	Ile
Val	Asp 130	Met	Leu	Val	Arg	Ser 135	Gly	Ala	Leu	Asp	Leu 140	Ile	Val	Ile	Asp
Ser 145	Val	Ala	Ala	Leu	Val 150	Pro	Arg	Ala	Glu	Ile 155	Glu	Gly	Glu	Met	Gly 160
Asp	Ser	His	Val	Gly 165	Leu	Gln	Ala	Arg	Leu 170	Met	Ser	Gln	Ala	Leu 175	Arg
Lys	Ile	Thr	Ser 180	Ala	Leu	Asn	Gln	Ser 185	Lys	Thr	Thr	Ala	Ile 190	Phe	Ile
Asn	Gln	Leu 195	Arg	Glu	Lys	Ile	Gly 200	Val	Met	Phe	Gly	Ser 205	Pro	Glu	Thr
Thr	Thr 210	Gly	Gly	Arg	Ala	Leu 215	Lys	Phe	Tyr	Ala	Ser 220	Val	Arg	Leu	Asp
Ile 225	Arg	Arg	Ile	Glu	Thr 230	Leu	Lys	Asp	Gly	Thr 235	Asp	Ala	Val	Gly	Asn 240
Arg	Thr	Arg	Val	Lys 245	Val	Val	Lys	Asn	Lys 250	Val	Ala	Pro	Pro	Phe 255	Lys
Gln	Ala	Glu	Phe 260	Asp	Ile	Leu	Tyr	Gly 265	Gln	Gly	Ile	Ser	Arg 270	Glu	Gly
Gly	Leu	Ile 275	Asp	Met	Gly	Val	Glu 280	Asn	Gly	Phe	Val	Arg 285	Lys	Ala	Gly
Ala	Trp 290	Tyr	Thr	Tyr	Glu	Gly 295	Asp	Gln	Leu	Gly	Gln 300	Gly	Lys	Glu	Asn
Ala 305	Arg	Asn	Phe	Leu	Lys 310	Asp	Asn	Pro	Asp	Leu 315	Ala	Asn	Glu	Ile	Glu 320
Lys	Lys	Ile	Lys	Gln 325	Lys	Leu	Gly	Val	Gly 330	Val	His	Pro	Glu	Glu 335	Ser
Ala	Thr	Glu	Pro 340	Gly	Ala	Asp	Ala	Ala 345	Ser	Ala	Ala	Pro	Ala 350	Asp	Ala
λla	Dro	ת ו ת	17-1	Dro	λΙα	Dro	Thr	Thr	71-	Tara	ת 1 ת	Thr	Tara	Co~	Tarc

355 360 365

Ala Ala Ala Lys Ser 370

<210> 4

<211> 372

<212> PRT

<213> Streptomyces ambofaciens

<400> 4

Met Ala Gly Thr Asp Arg Glu Lys Ala Leu Asp Ala Ala Leu Ala Gln 1 5 10 15

Ile Glu Arg Gln Phe Gly Lys Gly Ala Val Met Arg Met Gly Asp Arg 20 25 30

Ser Lys Glu Pro Ile Glu Val Ile Pro Thr Gly Ser Thr Ala Leu Asp 35 40 45

Val Ala Leu Gly Val Gly Gly Leu Pro Arg Gly Arg Val Ile Glu Val 50 55 60

Tyr Gly Pro Glu Ser Ser Gly Lys Thr Thr Leu Thr Leu His Ala Val 65 70 75 80

Ala Asn Ala Gln Lys Ala Gly Gly Gln Val Ala Phe Val Asp Ala Glu 85 90 95

His Ala Leu Asp Pro Glu Tyr Ala Gln Lys Leu Gly Val Asp Ile Asp 100 105 110

Asn Leu Ile Leu Ser Gln Pro Asp Asn Gly Glu Gln Ala Leu Glu Ile 115 120 125

Val Asp Met Leu Val Arg Ser Gly Ala Leu Asp Leu Ile Val Ile Asp 130 135 140

Ser Val Ala Ala Leu Val Pro Arg Ala Glu Ile Glu Gly Glu Met Gly 145 150 155 160

Asp Ser His Val Gly Leu Gln Ala Arg Leu Met Ser Gln Ala Leu Arg 165 170 175

Lys Ile Thr Ser Ala Leu Asn Gln Ser Lys Thr Thr Ala Ile Phe Ile 180 185 190

Asn Gln Leu Arg Glu Lys Ile Gly Val Met Phe Gly Ser Pro Glu Thr 195 200 205

Thr Thr Gly Gly Arg Ala Leu Lys Phe Tyr Ala Ser Val Arg Leu Asp 210 215 220

Ile Arg Arg Ile Glu Thr Leu Lys Asp Gly Thr Asp Ala Val Gly Asn 225 230 235 240

Arg Thr Arg Val Lys Val Val Lys Asn Lys Val Ala Pro Pro Phe Lys 245 250 255

```
Gln Ala Glu Phe Asp Ile Leu Tyr Gly Gln Gly Ile Ser Arg Glu Gly
Gly Leu Ile Asp Met Gly Val Glu His Gly Phe Val Arg Lys Ala Gly
        275
                            280
Ala Trp Tyr Thr Tyr Glu Gly Asp Gln Leu Gly Gln Gly Lys Glu Asn
                        295
Ala Arg Asn Phe Leu Lys Asp Asn Pro Asp Leu Ala Asn Glu Ile Glu
                    310
                                         315
Lys Lys Ile Lys Glu Lys Leu Gly Val Gly Val Arg Pro Glu Glu Pro
Thr Ala Thr Glu Ser Gly Pro Asp Ala Ala Thr Ala Glu Ser Ala Pro
                                345
Ala Val Pro Ala Pro Ala Thr Ala Lys Val Thr Lys Ala Lys Ala Ala
                            360
        355
Ala Ala Lys Ser
    370
<210> 5
<211> 1125
<212> DNA
<213> Streptomyces lividans
<400> 5
atggcaggaa ccgaccgcga gaaggccctg gacgccgcgc tcgcacagat tgaacggcaa 60
ttcqqcaaqq qcgcqgtcat gcgcatgggt gaccggacca acgagcccat cgaggtcatc 120
cegacegqqt ctacegeqct egacgtggcc cteggegteg gaggcatece gegtggeegt 180
gtcgtggagg tctacggccc cgagtcctcg ggcaagacga ccctgaccct gcacgcggtg 240
gegaacgege agaaggeegg eggeeaggte gegttegtgg acgeegagea egeeetegae 300
cccgaqtacq cgaagaagct cggtgtcgac atcgacaacc tgatcctgtc ccagccggac 360
aacggtgagc aggccctgga gatcgtggac atgctggtcc gctccggcgc cctcgacctc 420
atogtcatog actocgtogo ogogotogto cogogogogo agatogagog ogagatogogo 480
gacagccacg teggtetgca ggeceggetg atgagecagg ceetgeggaa gateaceage 540
gcgctcaacc agtccaagac caccgcgatc ttcatcaacc agctccgcga gaagatcggc 600
gtgatgttcg gctccccgga gaccacgacc ggtggccggg cactgaagtt ctacgcctcg 660
gtgcgactcg acatccggcg tatcgagacg ctgaaggacg gcaccgacgc ggtcggcaac 720
cgcacccgcg tcaaggtggt caagaacaag gtcgcgccgc ccttcaagca ggccgagttc 780
gacatcetet aeggeeaggg cateageege gagggeggte tgategacat gggegtggag 840
aacgqcttcg tccgcaaggc cggcgcctgg tacacgtacg agggcgacca gctcggtcag 900
qqcaaqqaqa acqcqcqcaa cttcctgaaq gacaaccccg acctggccaa cgagatcgag 960
aagaagatca agcagaagct gggcgtcggc gtgcaccccg aggagtcggc caccgagccc 1020
qqcqcgqacg ccgcctccgc cgccccggcc gacgccgcac cggcggtgcc cgcacccacg 1080
acceccaage ccaccaagte caaggeegeg gcagecaaga getga
<210> 6
<211> 1119
<212> DNA
<213> Streptomyces ambofaciens
<400> 6
atggcaggaa ccgaccgcga gaaggctctt gacgccgcac tcgcacagat tgaacggcag 60
ttcggcaagg gcgcggtcat gcgcatgggc gaccggtcga aggagcccat cgaggtcatc 120
```

```
ccqaccqqqt cgaccgcgct cgacgtggcc ctcggcgtcg gcggcctgcc gcgcggccgc 180
gtcatcgagg tctacggtcc ggagtcctcc ggtaagacga ccctgaccct gcacgccgtg 240
gcgaacgcgc agaaggccgg cggccaggtg gcgttcgtgg acgcggagca cgccctcgac 300
cccqaqtacq cccagaagct cggcgtcgac atcgacaacc tgatcctgtc ccagccggac 360
aacggtgage aggeeetgga gategtggae atgetggtee geteeggege eetegaeete 420
ategteateg acteegtege egegetegte eegegegegg agategaggg egagatgggt 480
qacaqccacq teggteteca ggcceggetg atgagecagg egeteeggaa gateaceage 540
qeqeteaace aqtecaagac cacegegate tteateaace ageteegega gaagategge 600
gtcatgttcg gctcccgga gaccacgacc ggtggccggg cgctcaagtt ctacgcctcg 660
gtgcgactcg acatccgacg catcgagacg ctcaaggacg gcaccgacgc ggtcggcaac 720
cgcacgcgcg tcaaggtcgt caagaacaag gtcgcgccgc ccttcaagca ggccgagttc 780
gacatectet aeggeeaggg cateageege gagggeggee tgategaeat gggegtggag 840
cacggetteg teegeaagge eggegeetgg tacaegtaeg agggegaeca geteggeeag 900
ggcaaggaga acgcgcgcaa cttcctgaag gacaaccccg acctcgccaa cgagatcgag 960
aagaagatca aggagaagct gggcgtcgga gtccgtcccg aggagccgac ggccaccgag 1020
teeggacegg acgeeggae ggeegaatee geaceggegg tgeeeggee egegacegee 1080
aaggtcacca aggccaaggc cgcggcagcc aagagctga
                                                                   1119
<210> 7
<211> 14
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<220>
<221> modified base
<222> (6)..(14)
<223> a, c, g, t, unknown or other
<400> 7
ggatgnnnnn nnnn
                                                                   14
<210> 8
<211> 18
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<220>
<221> modified base
<222> (1)..(13)
<223> a, c, g, t, unknown or other
nnnnnnnnn nnncatcc
                                                                   18
<210> 9
<211> 35
<212> DNA
<213> Artificial Sequence
```

<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 9 ggcatatggc ggacctgtca aagctctccg acagt	35
<210> 10 <211> 36 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 10 ggtctagatc ccagccgcgg tcggtcggca gtcccg	36
<210> 11 <211> 53 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 11 ggtctagact cgctgttcca ccccgacccc acgcgctcgg gcaccgcgca cca	53
<210> 12 <211> 48 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 12 ggtctagatc gcgcagcgcg gcggactcgt cgacggggc gaaggcgg	48
<210> 13 <211> 49 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 13 ggtctagacg gtctcgcgac gggaaacgcc gacggtgccg ccgttggaa	49
<210> 14	

```
<211> 51
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 14
ggtctagatc caccgcgaca ccggcggcga acgcgcggga gagcgcttcg c
                                                                    51
<210> 15
<211> 47
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 15
ggtctagagt cggtgcacct gggcaccgga gcacgccggg tgccctt
                                                                    47
<210> 16
<211> 43
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 16
ggtctagatc gtcgaagagc ctggtcgggc gctgcgcggt gta
                                                                    43
<210> 17
<211> 52
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 17
ggtctagacg acgcgcggcg ggctgcgccg caggcgccgg ccgaaccgcg gg
                                                                   52
<210> 18
<211> 41
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide
<400> 18
```

ggtctagatc ggccgtggtc gccggtgccg cctgctcggc t	41
<210> 19 <211> 46 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 19 ggtctagacg agccgatcgc gatcgtcggc atggcgtgcc ggctgc	46
<210> 20 <211> 58 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 20 ggtctagatc gtgcacggcc teggcggtgt eggcggcgag caccgeggcc egetecte	58
<210> 21 <211> 33 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 21 ggtctagagg cggtggccga cggcgcggtg gtt	33
<210> 22 <211> 46 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 22 ggtctagatc gtcacgaggg gtggtgcggt ccggcagcag ccagaa	46
<210> 23 <211> 39 <212> DNA <213> Artificial Sequence	
~220\	

<223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 23 ggtctagacg gctggttcta ccgggtcgac tggaccgag	39
<210> 24 <211> 39 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 24 ggtctagatc cggccggggc cgggcggcgg tgtaggact	39
<210> 25 <211> 35 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 25 ggtctagacc gcatcgtcac gaccgcgccg agcga	35
<210> 26 <211> 23 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 26 ggtctagatc ggcgtcgagg aaa 2	23
<210> 27 <211> 33 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 27 ggtctagaca gcgggactcc cgcccgggaa gcg	33
<210> 28	

<212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 28 gggctagctc tagatcatga attccctccg cccagccagg cgtc	44
<210> 29 <211> 36 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 29 ggtctagaat teggcaaggg egeeggteat gegeat	36
<210> 30 <211> 38 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide	
<400> 30 ggtctagatc tgcggcgtcg gccggggcgg cggaggcg	38